Rule 1.126 87. (new) The particle of claim 1, wherein the particle length is from 1 - 15 μm, the particle width is from 2 μm to 50 μm, and the lengths of said segments are from 50 nm to 15 μm.

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## REMARKS

Minor formal corrections have been made to the specification. No new matter has been introduced in these amendments. Support for the amendments to the claims is provided below.

Ent. Sum. OK 5-21-03

### Examiner Interview

Applicants' interview with the Examiner on November 20, 2002 is summarized in the Interview Summary of the same date. Applicants thank the Examiner for the courtesy extended in granting them the opportunity to discuss the case.

### Claim Rejections – 35 USC §112, second paragraph

Claims 1-36 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the Applicants regard as their invention because of the term "freestanding".

The term "freestanding" is explained and exemplified in the specification, e.g., at page 14, line 29 to page 15, line 8. In particular, the specification states that "[b]y 'freestanding' it is meant that nanobar codes that are produced by some form of deposition or growth within a template have been released from the template. Such nanobar codes are typically freely dispensable in a liquid and not permanently associated with a stationary phase. Nanobar codes that are not produced by some form of deposition or growth within a template (e.g., self-assembled nanobar codes) may be considered freestanding even though they have not been released from a template." The term encompasses particles that are synthesized using a template, as well as those that are not.

Regarding the Examiner's assertion that particles in contact with a solution are apparently not "freestanding," the specification at the cited passage further indicates that "[t]he term 'free standing' does not imply that such nanoparticles must be in solution (although they

may be) or that the nanobar codes can not be bound to, incorporated in, or a part of a macro structure. Indeed, certain embodiments of the invention, the nanoparticles may be dispersed in a solution, e.g., paint, or incorporated within a polymeric composition" (page 15, lines 4-8). Therefore, Applicants submit that the term freestanding is sufficiently definite. In order to expedite examination of the application, however, Claim 1 has been amended to require that the particles are "template-separated" rather than "freestanding." Applicants' amendment does not imply acquiescence to the Examiner's statements regarding the term "freestanding," and Applicants reserve the right to pursue such subject matter in subsequent applications.

This amendment has ample support in the specification. In certain preferred embodiments, the particles of the present invention are separated from a template that was used to direct their synthesis. For example, metals may be electrochemically deposited in the pores of an alumina membrane (template) and then released by dissolving the membrane in 0.5 M NaOH. See, e.g., page 16, lines 3-12; page 23, line 25. Embedded inside the alumina membrane, the particles are not "template-separated." The particles are "template-separated" if, and only if, they are separated from the alumina membrane. Such particles are typically freely dispensable in a liquid and not permanently associated with a stationary phase. The term "template-separated" does not imply that the particles must be in solution (although they may be) or that the particles can not be bound to, incorporated in, or a part of a macro structure. Indeed, certain embodiments of the invention, the nanoparticles may be dispersed in a solution, e.g., paint, or incorporated within a polymeric composition. See page 15, lines 1-8.

For additional support, see, e.g., page 53, lines 3-4 ("[T]he nanobar codes were released from the alumina membrane by dissolving the membrane in 0.5 M NaOH"); page 27, lines 7-8 ("[A] final critical step is required to separate each unique type of nanorod and release all the nanorods into solution"); page 27, lines 20-22 ("Etching fluid is then introduced into the channels which dissolves the Ag backing and carries the nanorods into the corresponding well. Other means for removing particles from the membrane are also possible, including laser ablation, controlled acid or base ablation, and so on."); page 7, lines 10-14 ("In preferred embodiments of the present invention, the nanobar code

particles are made by electrochemical deposition in an alumina or polycarbonate template, followed by template dissolution, and typically, they are prepared by alternating electrochemical reduction of metal ions, though they may easily be prepared by other means, both with or without a template material.").

Applicants believe that all claims are now definite and respectfully request that the rejections be withdrawn.

Claim 1 has also been amended to require that the particles have "2 to 50 segments" rather than "a plurality of segments." Support for this amendment can be found at, e.g., page 10, lines 10-11. None of the art of record discloses a template-separated particle comprising 2 to 50 segments, wherein the particle length is from 20 nm to 50 μm and the particle width is from 5 nm to 50 μm.

Claim 22 has been amended to correct the spelling of the word "polystyrene".

Support for new claim 37 can be found at, e.g., page 9, lines 28-30; page 50, line 10; claim 1 and 10.

**Claim Rejections – 35 USC §103(a)**

Claims 1-12, 14, 18-23, 27 and 29-34 were rejected under 35 USC § 103(a) as being obvious over U.S. Patent No. 4,053,433, issued to Lee.

Lee discloses "microparticles which are encoded with an orderly sequence of visually distinguishable colored segments" (abstract; col. 2, lines 30-32). The microparticles are used to tag substances and are decoded by visual inspection of the color sequence using a microscope or other magnifying device (col. 2, lines 15-17).

The particles of Lee are not synthesized using a template. Rather, the particles are made by non-template means such as cutting fused plastic films using a double skiving process (col. 8, lines 5-39). Accordingly, the particles of Lee are not "template-separated." Applicants therefore submit claims 1-12, 14, 18-23, 27 and 29-34 to be patentable over Lee.

Claims 18-36 of the application require that at least one segment of the particle is "functionalized." The Examiner states that, with respect to the particles of Lee, "color is seen via the reflectance of light from such particles and thus describes functionalization of such color practices for microparticles of Lee" (emphasis added). However, this does not make the particles "functionalized" as that term is used in the specification and claims of the present application.

The specification states that "[b]y functionalization, or attachment of a functional unit, it is meant that some species or material is covalently or noncovalently attached to the surface of the particle. Examples of functionalization include the attachment, often via a linker, to an antibody or antibody fragment, to an oligonucleotide or a to a detectable tag" (page 13, lines 9-15) (emphasis added). See also, page 4, lines 17-19 ("In certain preferred embodiments of the invention, the particles are 'functionalized' (e.g., have their surface coated with IgG antibody)."); page 8, lines 9-12 ("A second key feature of the bar code nanoparticles is that the full range of chemical surface functionalization can be applied to the nanoparticle surface, including, but not limited to, functionalization with self assembled monolayers (SAMs), polymers, oxides, other metals, nucleic acids, proteins, lipids and combinations thereof.").

Thus, the use of colors of particle layers in a coding scheme as described in Lee does not involve a "functionalized" particle. No species or material is disclosed or suggested by Lee as being covalently or noncovalently attached to the surface of the particles.

## Double Patenting (Obviousness-Type)

Claims 1-36 were provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-36 of copending patent application Serial No. 09/677,198 ("the '198 application"). Claims 1-86 of the '198 application were subject to restriction and/or election requirement. In a response dated September 4, 2002, Applicant elected to not to prosecute claims 1-36 of the '198 application; therefore, it is believed that the double patenting rejection is improper. Reconsideration is respectfully requested.

## Informality

The Examiner has objected that in Claim 22, the word "polystyrene" has been misspelled. Claim 22 has been amended to replace "polystyrene" with "polystyrene" in order to address the Examiner's objection.

Applicants request that the Examiner reconsider the application and issue a Notice of Allowance in the next Office Action. If it would be helpful to obtain favorable consideration of this case, the Examiner is encouraged to call and discuss this case with the undersigned.

This constitutes a request for any needed extension of time and an authorization to charge all fees therefor to deposit account No. 19-5117, if not otherwise specifically requested. The undersigned hereby authorizes the charge of any fess created by the filing of this document or any deficiency of fees submitted herewith to be charged to deposit account No. 19-5117.

Respectfully submitted,

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Marked-up version of the specification amendments:

Page 6, lines 8-9:

Figure 3 is an image taken from an optical microscope in reflected light mode of a 9-striped bar code [(Au-/Ag-/Au-/Ag-/Au-/Ag-/Au-/Ag-/Au)] (Au/Ag/Au/Ag/Au/Ag/Au/Ag/Au) of the present invention.

Page 6, lines 19-20:

Figure 6A is an image of a collection of [Ag-/Au-] Ag/Au nanorods at 400 nm and Figure 6B is an image of the same collection at 600 nm.

Page 9, lines 22-27:

The particles of the invention are frequently referred to as being "rod" shaped. However, the cross-sectional shape of the particles, viewed along the long axis, can have any shape, and can change at different portions of the particle. Such cross-sections may be a circle, an oval, square, diamond or even tubular. In preferred embodiments of the invention, the cross section is a circle and the particles are "rod" shaped. Although the particles of the present invention may take many shapes, the [sequential] segmented particles of the present invention are not spherical.

Page 16, lines 17-29:

A key property of certain embodiments of the particles of the present invention is that when the nanorods are segmented, differences in the reflectivities of the component metals can be visualized by optical microscopy. Thus, in a segmented [Au-/Pt-/Au] Au/Pt/Au rod of 200 nm in diameter and 4-5 microns in overall length, the segments are easily visualized in a conventional optical microscope, with the Au segments having a gold lustre, and the Pt segments having a more whitish, bright lustre. Another key property of the materials is that the length of the segments, when they are prepared by alternating electrochemical reduction of two or more metal ions, is controlled (and defined) completely by a) the composition of the solution and b) the number of Coulombs of charge that are passed in each step of an electrochemical reduction. Thus, the widths and the

number of the segments can be varied at will. Figure 5 shows an image of a collection of nanoparticles of the present invention comprised of six different types or flavors of nanoparticles. This image demonstrates the ability to differentiate between the different types of nanobar codes in a collection of nanobar codes.

Page 18, lines 9-14:

A unique characteristic of nanobar codes, is the ability to differentially modify their surfaces. Thus, considering [Au-/Pt] Au/Pt nanobar codes, each metal may be selectively modified, providing two different chemistries to be placed in close proximity. In the case of nanobar codes, the ability to put different molecules on the Pt and Au stripes of a single particle has been demonstrated. The ability to rationally modify selected parts of a nanoparticle is without precedent.

Page 23, line 21:

PREPARATION OF [METALIC] METALLIC SEGMENTED PARTICLES

Page 24, lines 13-21:

To generate two-segment nanobar codes, two metals (e.g., Au, Ag, Pd, Cu, etc.) can be electroplated sequentially or simultaneously to form alloys. Nanobar codes can also be generated using 3 different metals. Synthesis of a [Au-/Pt-/Au] Au/Pt/Au rod may be accomplished with 1 C of Au, 8 C Pt, and 1 C of Au. The nominal dimensions of the segments are 1 μm of Au, 3 μm of Pt, 1 μm of Au. The 5-segment nanobar codes, [Ag-/Au-/Ag-/Au-/Ag] Ag/Au/Ag/Au/Ag, were generated by sequentially plating and optionally rinsing the appropriate metal. In some embodiments it is possible to include all metals in solution but control deposition by varying the charge potential current A nine-segment nanobar code, [Au-/Ag-/Au-/Ag-/Au-/Ag-/Au-/Ag-/Au] Au/Ag/Au/Ag/Au/Ag/Au/Ag/Au seen in Figure 3 has also been prepared. The number of segments can be altered to desired specifications.

Page 28, lines 12-21:

The ability to make complex bar codes is of no consequence without an effective method for reading the bar codes. Fortunately, in the case of metallic bar codes of approximately 100 nm or more in width and about 2 microns to 15 microns in length, differences in metal segment reflectivities can be visualized using conventional light microscopy. Thus, is possible to distinguish (and quantify) the number of rods by visual inspection; such a task could be automated. It is also possible to distinguish segments of different lengths within individual bar codes. Images have been obtained of a 9-striped bar code [(Au-/Ag-/Au-/Ag-/Au-/Ag-/Au-/Ag-/Au)] (Au/Ag/Au/Ag/Au/Ag/Au/Ag/Au) in which the four Ag segments were grown to different lengths. See Figure 3. The image was obtained using an optical microscope in reflected light mode, using a 400 ± 40 nm bandpass filter to improve resolution and enhance image contrast.

Page 34, line 28 to page 35, line 7:

The ability to place two different chemistries on the same [Au-/Pt] Au/Pt nanobar code may also be used in this embodiment of the invention. This leads to two opportunities, self-referencing nanobar codes and dual-assay nanobar codes. Both opportunities stem from the fact that since it is possible to control which sets of stripes are within a single nanobar code, it is possible to control the position(s) on the particle from which fluorescence is observed. If, for example, a capture antibody is placed solely on the Au stripes and a sandwich immunoassay is carried out using a fluorescently-tagged secondary antibody, there should be no fluorescence emanating from the Pt stripes: the Pt stripes are acting as an internal standard. Thus, any fluorescence coming from the Pt must be ascribed to non-specific binding, and can be digitally subtracted. This principle can be demonstrated using matched and mismatched capture oligonucleotides on the different metals. This becomes a self-referencing nanoparticle.

Page 44, lines 24-29:

Cylindrically-shaped colloidal metal nanoparticles of the present invention will be used in which the metal composition can be alternated [(e.g., Pt-/Au-/Pt-/Au-/Pt)] (e.g., Pt/Au/Pt/Au/Pt) along the length, and in which the metal segments can be length-tuned.

These nanobar codes will serve as solid phase identity tags for the immunoassays to be developed. In a typical sandwich immunoassay, the capture antibody will be conjugated to a specific nanobar code and the corresponding detection antibody will be labeled with a fluorophore.

Page 55, lines 14-23:

Flow cytometry experiments have been employed to quantitate fluorescence from immunoassays or nanobar codes. Both human IgG and biotinylated Cc systems have been investigated. The rabbit IgG system was switched to the biotinylated Cc system because TR could not be excited with 488 nm in the flow cytometry instrument. Titration curves were prepared for the human IgG and the biotinylated Cc systems on [Au-/Ag] Au/Ag nanobar code. From the graphs, it appears that the titration curve for human IgG contains an inflection point, whereas the biotinylated Cc system does not. Instead, it reaches a maximum and appears to level off. The shape of the curve for the human IgG system may originate from Ag enhancement of FITC. Flow cytometry experiments may be conducted to determine the amount of antibody binding capacity (ABC), as well as the concentration of capture antibody needed to optimize the system.

Marked-up version of the claim amendments:

1. (amended) A [freestanding] template-separated particle comprising [a plurality of] 2 to 50 segments, wherein the particle length is from 20 nm to 50 µm and the particle width is from 5 nm to 50 µm.

10. (amended) The particle of claim 1,[ comprising 2 - 50 segments,] wherein the particle length is from 1 - 15 µm, the particle width is from 30 nm to 2 µm, and the lengths of said segments [is] are from 50 nm to 15 µm.

22. (amended) The particle of claim 21 wherein said organic material comprises carbon, charcoal, diamond or [polystyene] polystyrene.

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